STUDIES ON THE TOXICITY AND FUNGICIDAL EFFICIENCY OF SULPHUR DUSTS IN THE CONTROL OF SOME CEREAL RUSTS.

F. J. Greaney

Reprinted from Scientific Agriculture, Vol. VIII., No. 5, January, 1928

STUDIES ON THE TOXICITY AND FUNGICIDAL EFFICIENCY OF SULPHUR DUSTS IN THE CONTROL OF SOME CEREAL RUSTS.*

F. J. GREANEY

[Received for publication November 16, 1927]

INTRODUCTION.

One of the greatest problems in agriculture in Western Canada is the effective control of epidemics of cereal rusts. Federal and Provincial investigators are working intensively on the breeding and selection of rust resistant varieties. There is no question that the development of resistant varieties, which will be at the same time agronomically suitable and commercially desirable, would be the ideal solution to the rust problem. However, in view of the fact that the application of sulphur dust has given promise of some immediate relief in the control of cereal rusts this method of control seemed worthy of some attention as well. Laboratory and greenhouse experiments were carried out to determine more of the value of sulphur dusts in the control of some cereal rusts.

HISTORICAL.

Sulphur has long been known as an effective fungicide. As early as 1833, before there was any general use of fungicides, a mixture of lime and sulphur was used for controlling severe epidemics of the grape vine mildew in France. Lime sulphur is still recognized as one of the most valuable spray mixtures. It is in this form that sulphur is most widely used for fungicidal purposes. The abundance and low cost of sulphur as well as the diversity of its forms have been influential factors in popularizing the use of sulphur as a fungicide.

Sulphur dust was used by Smith *(8)* to control the rust of asparagus. Other workers, Butler *(4)*, and Stone *(9)*, proved its effectiveness in the control of snapdragon rust In 1924 Kightlinger *(7)* reported that sulphur dust controlled cereal rusts in some preliminary experiments undertaken in New York State. Field studies conducted by Bailey and Greaney *(1)* in 1925, afforded striking confirmation of Kightlinger's work. Extended field tests in 1926 by the same authors *(2)* indicated the effectiveness of sulphur dust in controlling stem rust under conditions of a natural epidemic.

Young *(14)* has made intensive studies on the toxic property of sulphur. He found that the toxicity was not due to sulphur dioxide, sulphur trioxide, or hydrogen sulphide, or any of the common acids or oxides of sulphur, or to the sulphur particle, but to pentathionic acid which is an oxidation compound formed from sulphur and water. This author gives a complete review of the earlier investigations on the toxic constituent of different forms of sulphur. Wallace, Blodgett and Hesler *(12)*, and Windisch *(13)* con-

* Contribution from Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

-luded that the fungicidal effect of sulphur is due to sulphur dioxide which is produced by oxidation of the sulphur in moist air. This toxic effect from pure sulphur dioxide has not been obtained by other investigators. Doran *(5)* showed that sulphur is toxic to spores of plant pathogenes only when oxygen is present, thus indicating that some oxidation product of sulphur is undoubtedly the toxic agent. Tisdale *(11)* reported studies on the methods of preparation as well as the toxicity of colloidal sulphur.

Young *(14)* and, also, Tisdale *(11)* demonstrated that colloidal sulphur was effective in inhibiting the germination of spores of certain economic fungi. The toxicity of various forms of sulphur was studied by several other workers. Doran *(5)* found that certain commercially manufactured forms of precipitated sulphur were more effective in killing spores of *Venturia inaequalis* than some other finely divided sulphurs. It was demonstrated by Barker, Gimingham, and Wiltshire *(3)*, that the growth of many fungi was entirely inhibited, in a suspension of flowers of sulphur, in Van Teighem cells. These writers found that the spore germination of *Fusicladium dentriticum,* and of *Cladosporium fulvum* were 50 per cent inhibited, while that of *Nectria ditissima, Botrytis cinerea,* and *Verticillium* sp., was not at all inhibited. Kightlinger *(7)* has also reported that a 90:10 sulphur lead arsenate dust has a very inhibitory effect on the germination of urediniospores of *Puccinia coronata.*

In 1925, Bailey and Greaney *(1)* carried out some preliminary field experiments on the control of leaf and stem rust of wheat by sulphur dust. In these trials Sulfodust was used. This dust controlled leaf and stem rust to a remarkable degree. Similar studies were made in 1926 with two sulphur dusts, Kolodust and Sulfodust*. Under field conditions, no obvious difference was observed in the fungicidal efficiency of these dusts.

Present Investigations.

Influence of Sulphur Dusts on Spore Germination.

A comparative study was made in the laboratory of the inhibitory effects of "Kolo" and Sulfo" forms of sulphur dust on the germination of urediniospores of *Puccinia graminis.* Spores, uniformly matured from cultures of *Puccinia graminis tritici,* f.21 and *Puccinia graminis avenae,* f.3, were used for all germination tests. These wheat and oat strains of *Puccinia graminis* were collected in Western Canada, and are very constant in their reaction on the differential hosts. A suspension of urediniospores was made of the particular strain to be tested. This consisted of .002 grams of spores thoroughly dispersed in 30 cubic centimeters of distilled water.

For germination chambers, petri dishes were fitted with blocks of filter paper, so that two microscope slides could be accommodated in each dish. A quantity of water sufficient to cover the bottom of each was added. A large drop of the spore suspension (approx. .3 cc.) was placed on each slide. Nine of these chambers prepared for each test were divided into three sets. One set was left undusted as a check. The covers of the second set were removed long enough to permit the slides to be dusted

* Manufactured by the Niagara Sprayer Company, Middleport, N.Y.

lightly with Kolodust. The third set of slides was similarly treated with Sulfodust. All the cultures were then placed in an incubator and held at a temperature ranging from 18° to 20°C. The same procedure was followed in preparing all of the germination tests.

Preliminary tests resulted in slight irregularities in urediniospore germination. As it is impossible to eliminate all such irregularities, a sufficiently large number of germination tests was made with each strain to furnish an adequate test of the viability of the spores under the various conditions. A count of 50 spores was made after 24 hours in four different sections of the drop on each slide. In each test, therefore, 1200 spores were counted from the six undusted slides. The same number of spores were counted on the six slides of each dusted set. Only those spores possessing germ tubes longer than the width of the spore were considered as having germinated.

Kolodust and Sulfodust were examined under the microscope to determine the fineness of particles of the sulphur. Kolodust is more finely divided than Sulfodust. The former dust is colloidal in nature. Under the conditions of the experiment both forms of sulphur dust were extremely toxic to urediniospores of *Puccinia graminis tritici* and *Puccinia graminis avenae*. In either case the spores on the dusted slides germinated but poorly, whereas on the undusted slides they developed vigorous germ tubes in all the counts. Table 1 shows that Kolodust was more toxic than Sulfodust, so these results conform with those of Young *(14)*, and Thatcher and Streeter *(10)*, who found that the toxicity of sulphur increased in proportion to the fineness of its particles.

TABLE 1.—*Influence of sulphur dusts on the germination of urediniospores of Puccinia graminis tritici and Puccinia graminis avenae*

Organism	Form of sulphur dust	Tests (a)						Aver.
		1	2	3	4	5	6	
P. gr. tritici, f. 21	Kolodust	2	3	2	1	1	2	1.8
	Sulfodust	5	10	10	7	3	10	7.5
	Without sulphur dust	56	85	89	87	91	85	82.1
P. gr. avenae, f. 3	Kolodust	10	6	9	2	2	1	5.0
	Sulfodust	24	18	21	10	24	11	18.0
	Without sulphur dust	83	68	91	93	91	90	86.0

(a) Each test from actual counts of 1200 spores.

FACTORS INFLUENCING THE EFFICIENCY OF SULPHUR IN CONTROLLING RUST

(a) Free moisture before and after inoculation.

Studies were made to determine what influence free moisture before and after inoculation, had on the effectiveness of Kolodust and Sulfodust in controlling stem rust.

Sufficient Marquis wheat seedlings for the experiment were grown in 5-inch pots, ten to twelve plants in each pot. Ten days after planting, when

the first leaves were from 8 to 10 centimetres long, the plants were divided into two series.

One of these series was again divided into three sets which were subjected to the following conditions. One set was dusted with Kolodust, the second with Sulfodust, and the third was left undusted. The dusted plants received a uniform light application of dust, which was applied by means of a small hand duster. A light sprinkle of water was applied each day to the dusted and undusted plants, in amount about the equivalent of a heavy morning dew. Immediately following the dust treatment, approximately fifty plants were inoculated from each set of the dusted plants and fifty from the undusted ones. Thereafter, at one-day intervals, up to and including the twelfth day, fifty plants were inoculated from each of the three sets of plants. These plants were inoculated by the ordinary needle method, that is, by moistening the lower leaf of each seedling and applying urediniospores from uniform stock cultures. *P. gr. tritici,* f.21 was used in these trials. When inoculated the plants were incubated in moist chambers for 48 hours, at a temperature of 20°-22°C. Separate chambers were used for each set of dusted and undusted plants in order to avoid any effect from gases. The plants were taken from the incubation chambers and placed on the centre bench in the greenhouse.

The second series of plants was divided into three sets and very similarly treated. Inoculations were made immediately after the dust applications and at corresponding intervals. However, in this series inoculations were made by applying the spores to the unmoistened leaves with a dry needle. The inoculated plants were incubated for 24 hours instead of 48 hours. The most important point was that these plants were not sprinkled during the entire period of the experiment. Both series of plants were grown at the ordinary greenhouse temperature. Final data on the percentage of plants infected in each series were recorded fourteen days after inoculation.

The summarized results of these studies are given in Table 2, and plotted in Figures 1 and 2. The fungicidal effectiveness of Kolodust and Sulfodust was greatly reduced when free moisture was abundant before and after inoculation. Under such conditions considerable infection occurred when only one day had elapsed between the time of dust application and inoculation, whereas, under relatively dry conditions, i.e. if the plants were not sprinkled, were dry at inoculation, and were incubated for only 24 hours, both sulphur dusts were effective for long periods. Under these conditions a very small percentage of the plants were infected even when twelve days elapsed between the dust application and inoculation. Kolodust was slightly more effective than Sulfodust. Photographs of dusted and undusted wheat plants inoculated with *P. gr. tritici,* and *Puccinia triticina* respectively, are shown in Plate 1.

A comparison was made also of the effectiveness of the two sulphur dusts in controlling stem rust when the dusted plants were subjected to a shower of water for various periods and then inoculated.

TABLE 2.—*Influence on rust control of water at the time of and subsequent to dusting with sulphur.*

Interval between dust application and time of inoculation	PERCENTAGE OF PLANTS INFECTED UNDER THE FOLLOWING EXPERIMENTAL CONDITIONS (a)					
	Plants sprinkled daily with water, wet at inoculation, 48 hours in moist chamber.			Plants not sprinkled daily, dry at inoculation, 24 hours in moist chamber.		
	PLANTS DUSTED WITH					
Days	Kolodust	Sulfodust	Check Undusted	Kolodust	Sulfodust	Check Undusted
0	1	1	99	0	0	58
½	8	18	98	0	0	57
1	29	43	94	1	3	68
2	41	44	97	0	3	75
3	52	54	100	0	3	75
4	93	92	100	0	5	76
5	80	74	94	5	3	74
6	81	91	91	0	2	69
7	51	65	82	4	3	55
8	91	85	96	0	7	75
9	48	80	97	0	0	70
10	73	91	93	4	5	67
11	63	82	88	5	7	65
12	93	97	96	2	5	77

(a) Average of two tests run at different times. Approximately fifty plants were inoculated in each test.

Fig. 1.

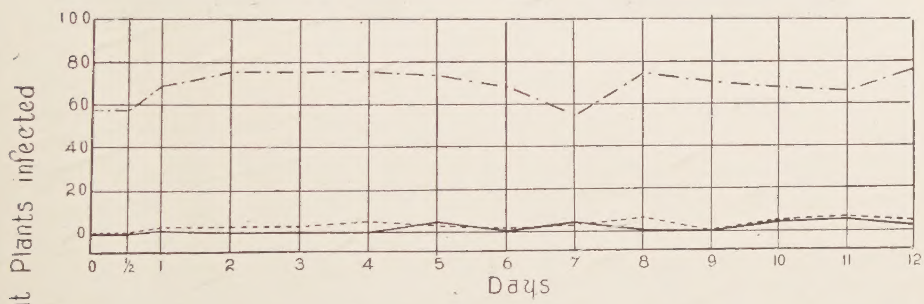

Fig 2

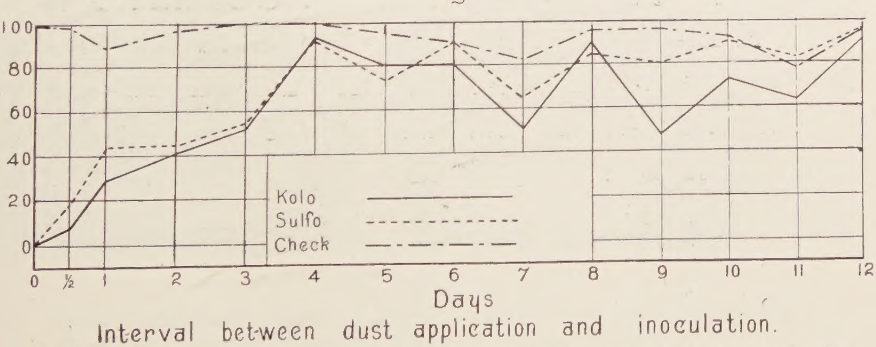

Figure 1.—The effectiveness of two sulphur dusts in the control of stem rust, when dusted Marquis wheat plants were not sprinkled with water, were dry at inoculation, and incubated for 24 hours.

Figure 2.—The effectiveness of two sulphur dusts in the control of stem rust when dusted Marquis wheat plants were sprinkled daily with water, were wet at inoculation, and incubated for 48 hours.

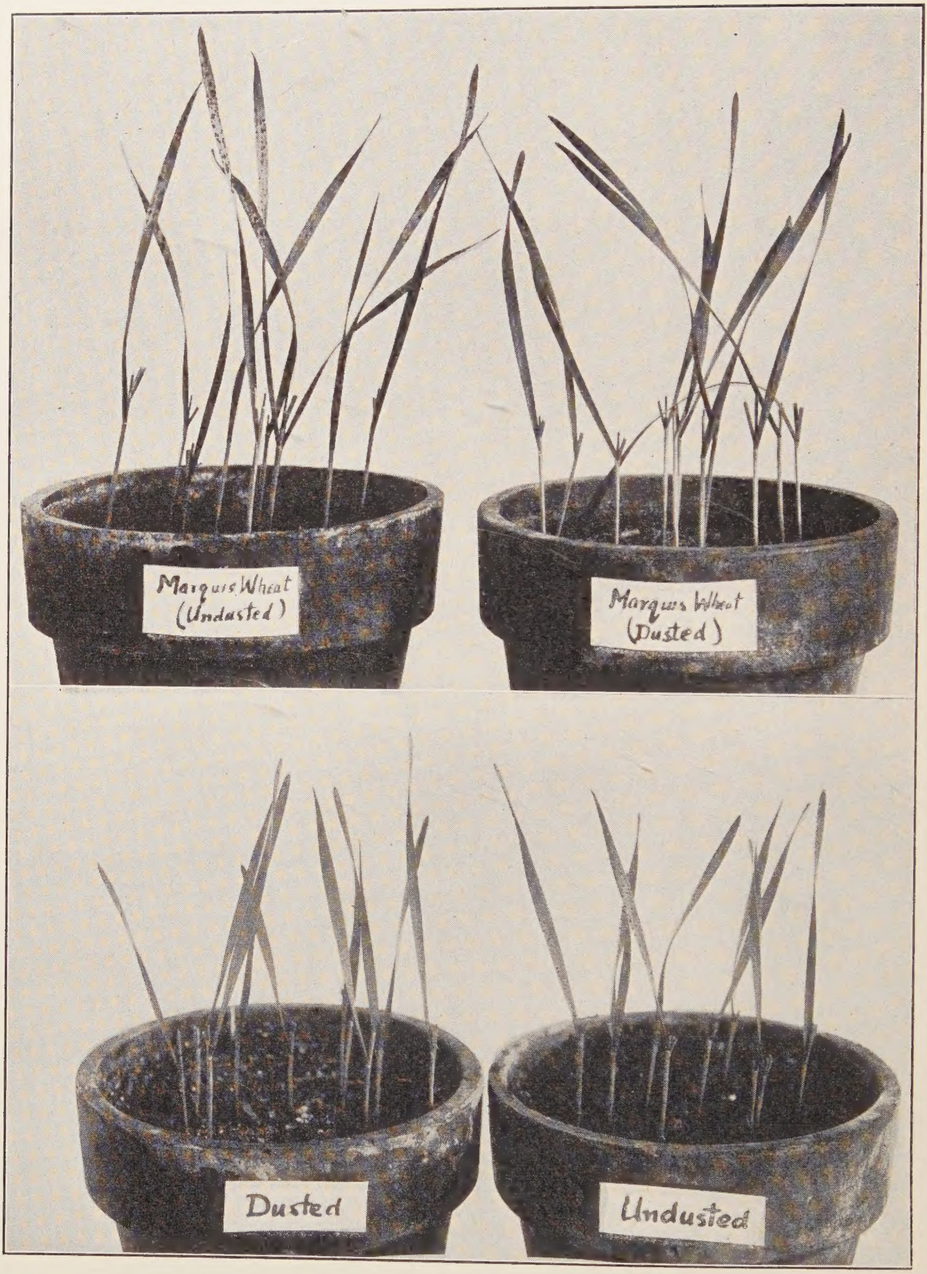

Plate I.

(Above) Effectiveness of sulphur dust in controlling stem rust of wheat. *P. gr. tritici.*

Marquis wheat plants 12 days after inoculation . Left—Undusted plants. Right—Dusted plants.

(Below) Effectiveness of sulphur dust in controlling leaf rust of wheat. *Puccinia triticina.*

Little Club wheat plants 12 days after inoculation. Left—Dusted plants. Right—Undusted plants.

Marquis wheat seedlings were grown in sufficiently large numbers in pots and divided into three series. One series was left undusted; the second was dusted with Kolodust; and the third with Sulfodust. Immediately following the application of dust all of the plants were placed under an artificial shower of water. This shower would correspond in intensity to an ordinary rain of similar duration to which plants would be subject under field conditions. Forty plants from each of the three series were removed after each of the following intervals, 1½, 3, 5, 10, 15, 30, 45, 60, and 120 minutes. Following this, the plants were inoculated by the moist needle method and incubated in moist chambers for 48 hours. In order to avoid further loss of sulphur from the dusted plants after their removal from the chambers, particular care was taken during the regular daily watering of the pots, that no water came in contact with the leaves. The infection data were recorded fourteen days after inoculation. The results of two tests run at different times are presented in Table 3 and plotted in Figure 3.

Under the conditions of the experiment fifteen minutes' exposure to a shower reduced markedly the effectiveness of sulphur in controlling stem rust of wheat. It will be noticed that Kolodust, owing probably to the fineness of its particles and to its greater ability to adhere, afforded the plants slightly greater protection than did the Sulfodust. Under the conditions of the experiment the sulphur did not adhere well to the plants.

Table 3.—*A comparison of the effectiveness of two sulphur dusts in the control of stem rust when dusted plants were exposed for different periods to a shower of water before inoculation.*

Period of exposure to shower of water in minutes.	PERCENTAGE OF MARQUIS SEEDLINGS INFECTED WHEN DUSTED WITH (a)		
	Kolodust	Sulfodust	Check (undusted)
0	0	1.3	97.4
1½	0	1.3	98.2
3	10.3	31.2	97.4
5	22.0	25.9	97.6
10	23.4	39.0	95.5
15	70.9	69.4	95.0
30	68.2	67.2	96.7
45	79.6	93.8	95.2
60	82.3	96.9	100.0
120	79.3	93.4	100.0

(a) Average of two tests run at different times. Approximately forty plants were inoculated in each test.

Fig. 3.

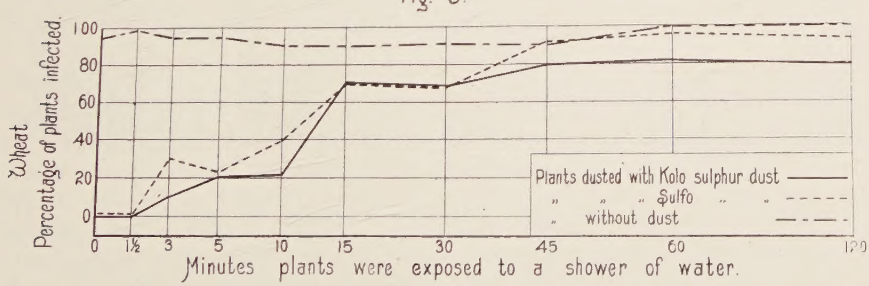

Figure 3.—A comparison of the effectiveness of two sulphur dusts in controlling stem rust when the dusted plants were exposed for different periods to a shower of water before being inoculated.

It seems probable that the fungicidal efficiency of sulphur dusts is increased in proportion to the fineness or smallness of particles, and that this is an important factor in the adherence of the material to the foliage.

(b)—Physical factors.

In comparing the fungicidal value of different sulphur dusts, the size of the particles seemed to be correlated with the efficiency. The more rapid oxidation of the smaller particles into toxic substances probably partially accounted for their greater efficiency, but it seemed possible that physical factors might be operating as well, for the finer dust seemed to absorb more water than the coarser ones. If this absorption were sufficiently pronounced, it might easily inhibit or retard germination by reducing the available moisture below the amount required for the rapid germination of the spores. In order to determine exactly how much weight should be attached to physical factors, a number of inert dusts of approximately the same fineness were used in comparison with Kolodust and Sulfodust, in spore germination and infection studies with *P. gr. tritici, P. gr. avenae,* and *P. triticina.* China clay ($H_4\ Al_2\ Si_2O_9$), Chalk ($CaCO_3$) and Talc ($H_2Mg_3\ Si_4O_{12}$) were used as inert materials because of their marked water-absorbing capacities Dusting was done before inoculation in one experiment and immediately after inoculation in another.

The data from these germination and infection studies are summarized in Tables 4 and 5. The inert dusts were not toxic to the growth of the urediniospores. There was no reduction in the percentage of spore germination as compared with the check cultures. Vigorous germ tubes were developed in all of the cultures dusted with the inert dusts whereas both forms of sulphur were extremely toxic. Infection studies indicated that the inert dusts were ineffective in reducing the amount of infection caused by the stem and leaf rust organisms. When sulphur was used a very marked reduction in the amount of infection was obtained. Photographs of the dusted and undusted plants are shown in Plate 2.

(c) Temperature.

Butler *(4),* found temperature an important factor in his experiments on the control of snapdragon rust with sulphur. Below 15°C., it was quite ineffective. Toxicity tests at different temperatures by Tisdale *(11)* with spores of *Botrytis cinerea* indicated that colloidal sulphur prepared from SO_2 and H_2S was most toxic at higher temperatures. An attempt was made to ascertain whether temperature had any appreciable influence in the present problem.

Soil temperature tanks were arranged to serve as moist incubation chambers. One set of chambers was maintained at a temperature range of from 10°-12°C. A second set was held at from 22°-25°C. One hundred and sixty Marquis wheat seedlings were inoculated with *P. gr. tritici,* f.21. One hundred of these were dusted immediately after inoculation, while sixty plants were left undusted as controls. Half of each of the dusted and undusted plants were held at the lower temperature while the remaining plants were incubated at the higher temperature. After 48 hours, all the plants were placed in sections of the greenhouse where approximately the temperatures at which incubation took place were maintained during the entire period of the experiment.

These results indicate that sulphur controls rust through the toxicity of some of its oxidation products and that the amount of moisture absorbed by the dust particles does not greatly influence its effectiveness.

A second experiment was performed which was exactly like the one already described except that the plants were dusted just before inoculation instead of immediately after. Under such conditions infection becomes more difficult, as a thin film of sulphur lies between the spores and the host. Each experiment was carried out at two different times.

The results of these experiments are summarized in Table 6. Under the conditions of the experiments there was no indication that temperature greatly influenced the fungicidal value of sulphur. Rust development was retarded at the lower temperature in all of the tests. A slight amount of infection occurred at 10°-12°C when the plants were inoculated before the dust was applied. In all cases, the pustules appeared at the tips of the leaves. During the incubation period, especially at the lower temperatures, large guttation drops are formed at the tips of the leaves. Besides reducing the toxic effect of the sulphur, the presence of abundant moisture at this part of the leaf undoubtedly induces more vigorous spore germination. It seems evident that a temperature as low as 12°C has very slight, if any, influence on the effectiveness of sulphur dust in controlling stem rust.

Plate II.

(Above) The effectiveness of various dusting materials in controlling stem rust of wheat. *P. gr. tritici.*

Fifteen-day old Marquis wheat plants dusted immediately after inoculation with—(Left to right) Talc, Chalk, Kolodust, China clay, Check (undusted).

(Below) The effectiveness of various dusting materials in controlling stem rust of oats. *P. gr. avenae.*

Fifteen-day old Victory oat plants dusted immediately after inoculation with—(Left to right) Arrangement same as in top figure.

TABLE 4.—*The effect of various dusts on the germination of urediniospores.*

Organism	Form of dust	TESTS (a)						
		1	2	3	4	5	6	Average
P. gr. tritici, f. 21	Kolo-sulphur dust	.6	1.1	.8	1.4	.4	.5	.8
	Sulfo-sulphur dust	2.5	4.3	5.1	4.0	2.8	6.6	4.2
	Chalk-$CaCO_3$	87	91	72	91	92	90	87.1
	China Clay $H_4Al_2\ Si_2O_9$	92	90	76	93	91	89	88.5
	Talc-$H_2Mg_3\ Si_4O_{12}$	86	89	81	88	89	92	87.5
	Check (without dust)	81	90	78	92	87	88	86.0
P. gr. avenae, f. 3	Kolo-sulphur dust	.4	.2	2.2	1.0	1.4	.3	.9
	Sulfo-sulphur dust	1.8	1.1	5.0	4.3	5.5	1.5	3.2
	Chalk-$CaCO_3$	86	75	92	90	76	74	82.1
	China Clay $H_4Al_2\ Si_2O_9$	82	61	94	90	60	88	79.1
	Talc-$H_2Mg_3\ Si_4O_{12}$	78	72	89	87	68	87	80.0
	Check (without dust)	78	71	96	94	68	83	81.7
Puccinia triticina	Kolo-sulphur dust	1.6	.8	.3	.6	1.1	.8	.9
	Sulfo-sulphur dust	2.6	3	1.1	2.1	2.1	3.1	2.3
	Chalk-$CaCO_3$	61	85	85	39	75	81	71.0
	China Clay $H_4Al_2\ Si_2O_9$	76	83	85	63	73	80	76.6
	Talc-$H_2Mg_3\ Si_4O_{12}$	85	86	89	53	73	78	77.3
	Check (without dust)	96	76	93	54	70	76	75.0

(a) Each test from actual counts of 1200 spores.

TABLE 5.—*The relative effect of various dusts on the subsequent rust infection in some greenhouse trials.*

Form of dust	TREATMENT OF PLANTS					
	Dusted before inoculation			Dusted immediately after inoculation		
	Percentage of plants infected when inoculated with (a)					
	P. gr. tritici	P. gr. avenae	P. triticina	P. gr. tritici	P. gr. avenae	P. triticina
Kolo-sulphur dust	1	2	0	4	24	15
Sulfo-sulphur dust	1	4	1.4	9	29	22
Chalk-$CaCO_3$	98	100	97	99	100	99
Talc-H_2Mg_3 Si_4O_{12}	98	98	100	96	100	98
China Clay H_4Al_2 Si_2O_9	98	99	99	96	100	100
Check (without dust)	97	100	100	99	100	100

(a) Average of two tests run at different times.
Fifty or more than fifty plants were inoculated in each test.

TABLE 6.—*The influence of temperature upon the fungicidal effectiveness of sulphur dust.*

Host and Pathogene	Treatment	Temperature Range Degrees C	Length of incubation period in days	INFECTION RESULTS			
				Dusted Plants		Undusted Plants	
				Percentage of plants infected (a)	Degree of infection (b)	Percentage of plants infected (a)	Degree of infection (b)
Marquis Wheat *P. gr. tritici*, f. 21	PLANTS DUSTED BEFORE INOCULATION	10° – 12°	13	0	0	83	Moderate
		22° – 25°	6	0	0	90	Light to heavy
	PLANTS DUSTED IMMEDIATELY AFTER INOCULATION	10° – 12°	12	3.8	Tip to light	78	Light to moderate
		22° – 25°	6	0	0	92	Heavy

(a) Average of two tests run at different times.
(b) Symbols indicating degree of infection.
tip—Pustules only at tip of leaf.
light—Pustules scattered, light infection.
moderate—Pustules numerous, general infection.
heavy—Pustules very abundant, heavy infection.

(d) Effect on Pustule Development.

Experiments were undertaken to determine what influence sulphur dust had on the development of rust pustules when the sulphur was applied too late to prevent infection.

Large numbers of wheat seedlings were grown and inoculated with *P. gr. tritici,* f.21. Immediately following the inoculation and at one day intervals up to, and including the tenth day, fifty plants were dusted with Kolodust. Fifty plants were left untreated for checks. Fourteen days after inoculation final data were obtained on the development of the pustules.

The results are summarized in Table 7. No infection occurred on plants dusted immediately after they were inoculated with *P. gr. tritici.* Where the interval between inoculation and dusting was one day or more, no control was achieved and the percentage of infected plants did not vary significantly whether this interval was 2, 4, 6, 8, or 10 days. However, the sulphur did retard the development of the individual pustules where it had been applied within three days after the inoculation of plants, but it did not influence to any significant degree the type of infection which finally developed. It is probable that repeated applications of sulphur after the plant has become infected would significantly affect the final type of infection.

TABLE 7.—*The effect of sulphur on pustule development of P. gr. tritici.*

	INFECTION RESULTS		
Interval between inoculation and time of dust application (days)	Percentage of plants infected (a)	Degree of infection (b)	Pustule development
0	0	0	No flecks
1	89	Tip to light	Retarded
2	98	Light to moderate	Retarded
4	99	Heavy	Slightly retarded
6	96	Moderate to heavy	Normal
8	100	Heavy	Normal
10	99	Heavy	Normal
Check (Undusted)	100	Heavy	Normal

(a) Average of tests run at two different times. Fifty or more than fifty plants were inoculated in each test.
(b) Symbols for degree of infection (See table 6).

(e) Influence on infection of the interval between inoculation and dusting when conditions are optimum for rust development.

In previous experiments it was observed that the effectiveness of sulphur was influenced by the length of time that intervened between the application of the sulphur and inoculation. Experiments were carried out to determine how soon after inoculation sulphur had to be applied in order to control rust effectively.

A large number of Marquis wheat seedlings were inoculated with *P. gr. tritici,* f.21, and were placed at once in moist chambers for 48 hours. Fifty plants were dusted immediately after inoculation with Kolodust. The same number of plants were dusted subsequently at intervals ranging from 1 to 48 hours after inoculation. Fifty inoculated plants remained untreated for controls. The plants were taken from the incubation chambers and held under uniform conditions of light and humidity in the greenhouse. The

greenhouse temperature throughout the experiment ranged from 20°-24°C. Ten days after inoculation the percentage of plants infected with rust was recorded. Final data were obtained at the end of 15 days. Two tests were made at different times.

The experiment was repeated using Victory oats and *P. gr. avenae,* f.3, instead of Marquis wheat and *P. gr. tritici,* f.21.

The results of these experiments are summarized in Table 8. It was found that rust was satisfactorily controlled if the sulphur was applied within six hours following inoculation. Where the interval was twelve hours or more, however, sulphur was quite ineffective. The results are what would be expected under the conditions of the experiment, for as soon as the plants were inoculated they were placed in moist chambers where conditions were ideal for the germination of the rust spores. Consequently within twelve hours, penetration into the plant would have been accomplished and the rust organism would then be beyond the influence of the fungicide.

TABLE 8.—*Percentage of plants infected when different intervals elapsed between the time of inoculation and of sulphur dust application.*

Time elapsing between inoculation and sulphur dust application	HOST AND PATHOGENE			
	Marquis Wheat—P. gr. tritici		Victory Oats—P. gr. avenae	
Hours	Percentage of plants infected (a)	Degree of infection (b)	Percentage of plants infected (a)	Degree of infection (b)
0	1	tip	0	0
1	3	tip to light	8	tip to light
3	3	tip to light	16	tip to light
6	14	tip to moderate	23	tip to moderate
12	85	light to heavy	74	tip to moderate
18	89	moderate to heavy	86	moderate
24	92	moderate to heavy	100	heavy
48	97	heavy	99	heavy
Check (Undusted)	100	heavy	100	heavy

(a) Average of two tests run at different times. Fifty or more than fifty plants were inoculated in each test.
(b) Symbols for degree of infection (See table 6).

Further experiments were carried on to determine what influence different temperatures might have on the length of period of effectiveness of sulphur following inoculation of plants and incubation in moist chambers.

Marquis wheat seedlings were inoculated with a spore suspension of *P. gr. tritici,* f.21. Half of them were placed in each of two moist chambers maintained at temperatures of from 10° to 12°C and from 22° to 24°C respectively. Beginning with the time of inoculation and at one hour intervals thereafter until the fourteenth hour, forty plants were taken from each incubator and dusted with Kolodust. After being dusted they were returned to the incubators to complete an incubation of 48 hours. For controls forty plants were inoculated and incubated at each temperature without being dusted. After the incubation in moist chambers, all of the plants were kept at the normal greenhouse conditions which prevailed at that time.

The results are summarized in Table 9. At the lower temperature (10°-12°C) sulphur prevented any serious infection for a period of 10 hours following inoculation, while at the higher temperature (22°-24°C) it was equally effective for only 5 hours. In each case the efficiency rapidly diminished after these periods had elapsed. In the lower temperature series the percentage of plants that became infected at each interval was lower

than the percentage infected at the corresponding interval of the higher temperature series. This is probably accounted for by the slower development of the rust fungus at the lower temperature, rather than by an increase in activity of the sulphur. The data contained in Tables 8 and 9 are presented graphically in Figures 4 and 5.

TABLE 9.—*Percentage of plants infected at different temperatures after different intervals have elapsed between inoculation and sulphur dust application.*

Interval between inoculation and time of sulphur dust application	RANGE OF TEMPERATURE			
	10 — 12°C		22 — 24°C	
Hours	Percentage of plants infected (a)	Degree of infection (b)	Percentage of plants infected (a)	Degree of infection (b)
0	0	0	0	0
1	0	0		” 83
2	0	0	0	0
3	4.4	tip	4	tip to light
4	7.5	tip to light	9	“ “
5	9	“ “	4.5	“ “
6	10	“ “	4.5	“ “
7	7	light	28	light
8	9	“	40	“
9	23	“	31	“
10	17	“	48	“
11	30	light to moderate	73	moderate
12	47	light	92	heavy
13	45	“	84	light to moderate
14	67	“	95	heavy
Check (undusted)	93	moderate	100	“

(a) Average of two tests run at different times. Approximately forty plants were inoculated and dusted in each test.
(b) Symbols for degree of infection (See table 6).

Fig. 4.

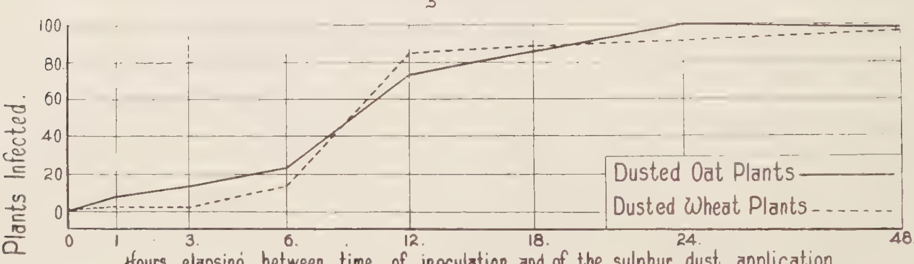

Fig 5.

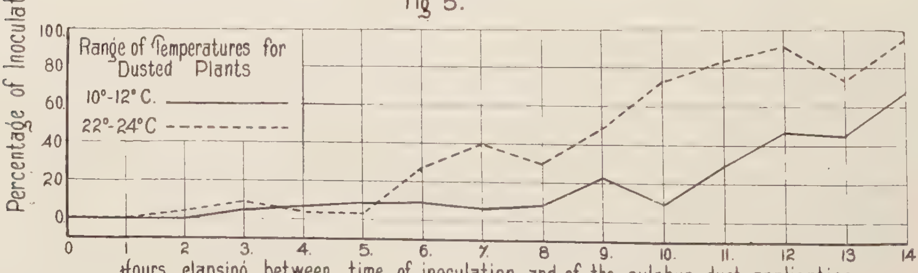

Figure 4.—Influence of sulphur on the control of wheat and oat stem rust, when different intervals elapsed between the time of inoculation and dust application.

Figure 5.—Influence of sulphur dust on wheat infection at different temperatures after different intervals elapsed between time of inoculation and dusting.

Discussion.

The preceding greenhouse and laboratory studies have yielded data of considerable practical importance. A comparison of the effectiveness of different sulphur dusts in controlling rust, indicated the importance of using very finely divided sulphur for the most effective control of stem rust. The commercial sulphur dusts used in these experiments were effective fungicides for long periods under relatively dry atmospheric conditions. The period of effectiveness of a single application of sulphur was greatly shortened if humid conditions prevailed. Therefore in practical use, the time of application and the total number necessary will be governed largely by weather conditions. Temperature influenced the fungicidal effectiveness of sulphur dusts less than did humidity.

From the results obtained it is evident that one of the most important factors in the control of rust by the use of sulphur dust is the time of application. Almost perfect control was obtained when plants were dusted before they were inoculated, provided the plants remained dry until inoculation. When the dust was applied after inoculation, the results were not so good, especially if the conditions prevailing between inoculation and dusting were favorable for infection. The data both for wheat and oat strains of stem rust and for leaf rust of wheat are comparable. Field studies of 1925 and 1926 indicated the desirability of making the initial application of sulphur dust very early. Every attempt should be made to have the plants dusted before rust appears in the field.

Dusting with sulphur constitutes an effective means of reducing the ravages of rust. Many practical difficulties incidental to applying the sulphur dust over large areas are still to be solved. Already however, this method of control should prove most beneficial in experimental plot work, where protection from rust injury is essential for securing vigorous seed lots, and also where seed is grown for registration.

Summary.

Experiments were carried out to determine the fungicidal value of two sulphur dusts in the control of some cereal rusts.

Spore germination tests to determine the toxicity of sulphur are described. Sulphur is extremely toxic to the germination of urediniospores. The toxicity is increased by the fineness of particles of the sulphur.

The fungicidal effectiveness of sulphur dust was studied under different conditions of humidity and temperature. High humidity greatly reduced the effectiveness of sulphur dust. Temperature appears to influence its effectiveness less than humidity.

The effectiveness of sulphur in reducing rust infection seems to be entirely due to the chemical properties of the sulphur. It is not significantly influenced by the physical changes which are brought about by the presence of the finely divided material on the dusted foliage.

The results obtained in these experiments indicate that one of the most important factors in the control of cereal rusts by the use of sulphur dust

is the time of application. Dusting should be done before inoculation occurs.

Dusting with sulphur constitutes an effective means of controlling rust diseases of cereals.

Dominion Rust Research Laboratory,
Manitoba Agricultural College,
Winnipeg, Man.

LITERATURE CITED

1. Bailey, D.L. and Greaney, F.J. Preliminary experiments on the control of leaf and stem rust of wheat by sulphur dust. Scient. Agric. *6:* 113-117. 1925.
2. ——————. Field experiments on the control of stem rust by sulphur dust. Scient. Agr. *7:* 153-156. 1927.
3. Barker, B.T.P., Gimingham, C.T., and Wiltshire, S.P. Sulphur as a fungicide. Univ. Bristol Agr. and Hort. Res. Sta. Ann. Rept. 1919: 57-75. 1920.
4. Butler, O. Experiments on the field control of snapdragon rust together with a description of a method for the control of the disease in the greenhouse. New Hampshire Agr. Exp. Sta. Tech. Bul. 22: 1-14. 1923.
5. Doran, W.L. Controlling snapdragon rust; value of copper and sulphur. Florists' Exchange *43:* p. 501. 1917.
6. —————— Laboratory studies of the toxicity of some sulphur fungicides. N.H. Agr. Exp. Sta. Tech. Bul. 19: 1-11. 1922.
7. Kightlinger, C.V. Preliminary studies on the control of cereal rusts by dusting. Phytopath. *15:* 611-613. 1925.
8. Smith, R.E. Asparagus rust control. California Agr. Exp. Sta. Bul. 172: 1-21. 1906.
9. Stone, R.E. Plant diseases in Ontario. Ont. Agr. Coll. and Exp. Farm Rept. 43: 20-31. 1918.
10. Thatcher, R.W. and Streeter, Leon R. The adherence to foliage of sulphur in fungicidal dusts and sprays. New York Agr. Exp. Sta. Tech. Bul. 116: 18p. 1925.
11. Tisdale, Lionel E. Colloidal sulphur: Preparation and toxicity, Ann. Mo. Bot. Garden *12:* 381-417. 1925.
12. Wallace, E. Blodgett, F.M., and Hesler, L.R. Studies of the fungicidal value of lime sulphur preparations. Cornell Univ. Agr. Exp. Sta. Bul. 290: 163-208. 1911.
13. Windisch, K. Untersuchung über die Wirkungsweise und Beschaffenheit des zur Bekampfung des Oidiums dienen Schwefels. Landw. Jahrb., *30:* 447-495. 1901.
14. Young, H.C. The toxic property of sulphur. Ann. Mo. Bot. Garden *9:* 403-435. 1922.